

Preparation of mini-Gs heterotrimer

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 An abbreviated version of this protocol was published in eLIFE in May 2018

Cryo-EM structure of the adenosine A_{2A} receptor coupled to an engineered heterotrimeric G protein

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Detailed protocol

The mini-G_S construct (399) used in single particle cryo-EM reconstructions is based on the construct 393 that was used in the structure determination of the A_{2A}R- mini-G_S crystal structure ([Carpenter et al., 2016](#); [Carpenter and Tate, 2016](#)). However, unlike construct 393, mini-G_S399 binds βγ ([Nehmé et al., 2017](#)). The expression and purification of the respective components and assembly to make the complex containing mini-G_S-β₁γ₂, and the preparation of nanobody Nb35, were all performed following the protocols described previously ([Carpenter and Tate, 2016](#); [Rasmussen et al., 2011](#); [Carpenter and Tate, 2017b](#)).

How to cite: (Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Tate, C. G.(2019). Preparation of mini-Gs heterotrimer. Bio-protocol Preprint. bio-protocol.org/prep77.
2. García-Nafria, J., Lee, Y., Bai, X., Carpenter, B. and Tate, C. G.(2018). Cryo-EM structure of the adenosine A_{2A} receptor coupled to an engineered heterotrimeric G protein. eLIFE. DOI: [10.7554/eLife.35946](https://doi.org/10.7554/eLife.35946)

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